American Journal of Medical Genetics 68:29–38 (1997)

Molecular Cytogenetic Identification of Four X Chromosome Duplications

Aiwu Zhang, David D. Weaver, and Catherine G. Palmer*

Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis

Four cases with previously unidentified X-chromosome abnormalities were studied by standard cytogenetic techniques and FISH in order to demonstrate the origin of the extra segment on the abnormal X chromosomes. All cases were identified as X-chromosome duplications by using a chromosome-specific painting probe. Application of appropriate locus-specific DNA probes as an adjunct to GTG- and RBG-banding proved useful in defining the breakpoints and the extent of the duplications. Although the duplicated X chromosome in female cases was selectively inactivated, as demonstrated by its late-replicating pattern, abnormal clinical findings were manifested in 3 female patients. Am. J. Med. Genet. 68:29–38, 1997 © 1997 Wiley-Liss, Inc.

KEY WORDS: duplications; X chromosome; X-FISH

INTRODUCTION

X-chromosome duplications have been identified in males and females. Clinical effects, readily ascertained in males with such duplications, may be observed in females as well. We describe here 4 patients with X duplications, 3 of which were ascertained in abnormal females, and 1 in a male. Studies in these cases included standard GTG banding, replication studies in females, and FISH studies with chromosome painting and unique DNA sequence probes.

MATERIALS AND METHODS

Patient 1

This case (family #89748) (Fig. 1a) was ascertained prenatally, following observation of meningomyelocele and hydrocephaly by ultrasound. Chromosome studies of cells from amniotic fluid showed a 46,XXq+ karyotype. At birth, which was at 38 weeks of gestation, the patient had hydrocephalus, a meningomyelocele, and bilateral clubfoot deformity. She also had respiratory distress which required intubation and ventilation, hyperbilirubinemia which necessitated phototherapy, and feeding difficulty which required gastric tube placement. At age 4 months, she was developmentally delayed, unable to roll over or to support her head. On examination, she had a prominent forehead, plagiocephaly, small and upturned nose with a depressed nasal bridge, nevus flammeus over the lower spine, and tapering fingers.

Patient 2

This family (family #83997) (Fig. 2) was originally ascertained in 1987 when a 3½-month-old white boy was referred for failure to thrive. The child had mild dolichocephaly, closed anterior fontanel, Brushfield-like spots in the irises, mild dystopia canthorum, epicanthal folds, mild retrognathia, asymmetric philtrum, clinodactyly, camptodactyly of the fifth fingers, and camptodactyly of the fourth left toe. He had extra skin folds on the scrotum. At age 5½ months, his length, weight, and head circumference (OFC) were 58.5 cm (3rd centile), 4.5 kg (3rd centile), and 37.7 cm (−2 SD), respectively. At this time, a 46,Xp+,Y chromosomal anomaly was diagnosed, and his mother and grandmother were found to carry the same abnormal X chromosome. A second son who was mildly retarded with seizures had normal 46,XY chromosomes. Six years later a 3-month-old son of the mother's sister was referred with growth deficiency and multiple congenital anomalies. The pregnancy of this mother [G(2)P(1)A(1)] had been complicated by low alphafetoprotein (AFP) and intrauterine growth retardation. This white boy (Fig. 1b) was born at term with respiratory distress and low birth weight. At age 3 months the patient had a weight of 2.8 kg (<3rd centile), a length of 49.5 cm (<3rd centile), an OFC of 34.5 cm (−3 SD), and multiple congenital anomalies including microcephaly, simple helices, short nose, thin lips, "carp-shaped" mouth, micrognathia, clinodactyly of the fifth digits, and syndactyly of the second and third toes, bilaterally. Genital abnormalities included undescended right testis and shawl scrotum. In addition, he had failure to thrive, bilateral hearing loss, strabismus, optic nerve atrophy of the right eye, and hypertonia. An EEG was abnormal,

*Correspondence to: Catherine G. Palmer, Ph.D., Cytogenetics Laboratory, Indiana University School of Medicine, 975 West Walnut Street, IB 264, Indianapolis, IN 46202-5251.

Received 2 October 1995; Accepted 5 April 1996.

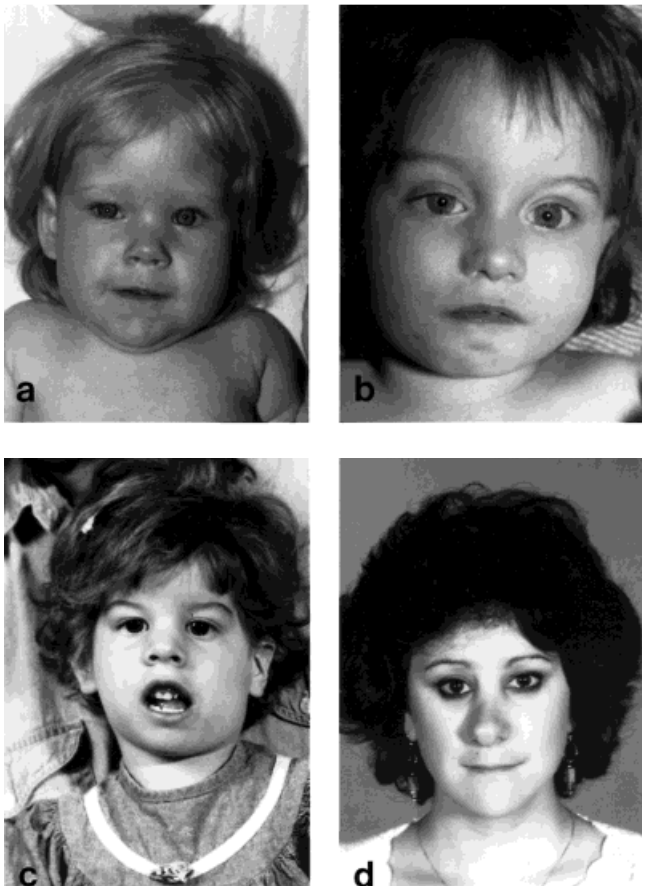

Fig. 1. **a**: Patient 1. **b**: Patient 2. **c**: Patient 3. **d**: Patient 4.

showing diffuse, slow waves. The mother of the patient was reported to have had low birth weight (4 lb, 2 oz). She also had learning disabilities and some minor anomalies such as bilateral clinodactyly of the fifth digits, "depressed" chin, and small stature.

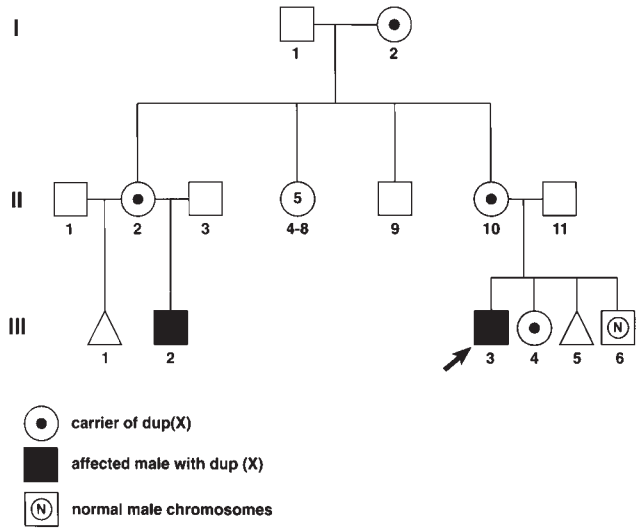

Fig. 2. Pedigree of patient 2.

Patient 3

This 29-month-old girl (family #87880) (Fig. 1c) was referred for developmental delay, short stature, and minor abnormalities. At birth she was small for gestational age and had respiratory distress. At age 29 months, her motor development was at the 18-month level but her social skills were appropriate for age. Her length and weight were 74.9 cm (<3rd centile) and 7.6 kg (<3rd centile), respectively. Other clinical findings included bitemporal narrowing, hypoplastic upper helices, "carp-shaped" mouth, proximally-placed thumbs, tapered fingers, clinodactyly of the fifth fingers, and slightly hyperconvex fingernails. Metacarpals were of normal length.

Patient 4

A 30-year-old white woman (family #88014) (Fig. 1d) was referred following two miscarriages and a stillbirth, the latter described as having Potter sequence. The patient had mild mental deficiency and had been in special classes throughout her education. She was small, with a height of 152.5 cm (5th centile), weight of 58 kg (25th centile), and OFC of 50.3 cm (−3.5 SD). In addition, she had malar flattening, left upslanted palpebral fissure, deviated nose, crowded teeth, pointed lower lateral incisors, very small hands and feet, hallux valgus, premature graying, and folliculitis on her arms, back, and legs. Her mother had had three miscarriages.

Cytogenetic/Molecular Cytogenetic Studies

Standard chromosome analysis with GTG-banding was performed on amniocytes from patient 1, on peripheral blood lymphocytes from all 4 cases, and from the parents of patients 1 and 3, the mother of patient 4, and relatives of patient 2, as well as from cells of a lymphoblastoid cell line (LCL) derived from patient 2's mother.

An R banding (RBG) procedure [Latt et al., 1976] was used to study replication of X chromosomes in patients 1, 3, and 4, and in patient 2's mother and grandmother. At least 20 cells were observed in each.

Studies of fluorescence in situ hybridization (FISH) were performed using standard techniques, with a whole-chromosome painting (WCP) probe for the X chromosome (Oncor, Gaithersburg, MD; or Cambio, Cambridge, England), as well as unique DNA sequence probes XIST (Xq13.2) and STS (Xp22.32) (Oncor), RC8 (Xp22.2) and DMD (Xp21) (ATCC, Brookville, MD), and Cos 9 (provided by Niklas Dahl, Uppsala, Sweden, who localized it to Xq25 by somatic cell hybrids). In order to improve specificity of hybridization, chromosome in situ suppression (CISS) hybridization was performed [Pinkel et al., 1988].

Hybridized cells were examined under a Leitz Aristoplan microscope (E. Leitz, Weltzlar, Germany) with a fluorescence filter with a wavelength range of 450–490 nm that allowed the fluorescence from FITC (green) and propidium iodide (PI) (red) to be visualized simultaneously. At least 20 cells were counted for each case, and photographs were taken with Kodak ASA 400 color slide film.

RESULTS

Patient 1

Chromosome analysis from amniocyte and lymphocyte cultures demonstrated a 46,X,Xq+ karyotype (Fig. 3a). Both parents had normal karyotypes, and thus the extra material was suggested to be a de novo rearrangement. Replication studies showed the abnormal X chromosome to be late-replicating, and the normal X chromosome early-replicating in all cells examined.

FISH with the whole-chromosome X-painting probe (WCP) performed on metaphase chromosomes showed uniform labelling along the entire length of both the normal and abnormal X chromosome (Fig. 4a). The chromosomal origin of the extra material was identified as from the X chromosome. GTG banding suggested that the duplicated segment originated in Xq. FISH with unique-sequence DNA probe XIST (Xq13.2) was performed on metaphase chromosomes. Only a single signal was present in each X chromosome in all cells (Fig. 5a). This suggests that the XIST locus was not included in the duplicated segment and that the breakpoint was distal to the XIST locus. In combination with GTG- and RBG-banding, the karyotype of patient 1 was defined as 46,X,dup(X)(q22.1→q13.3).

Patient 2

Chromosome analysis from lymphocyte cell cultures of the original proband (III-3, Fig. 2) and his mother showed a karyotype of 46,Xp+,Y and 46,X,Xp+, respectively (Fig. 3b). The abnormal male cousin (III-2, Fig. 2) had the same chromosomal abnormality. Four female relatives (I-2, II-2, II-10, and III-4) were found to be carriers of the abnormal X chromosome, and one male cousin (III-6) had normal 46,XY chromosomes. Replication studies of the lymphocytes of the maternal grandmother (I-2) and of the lymphoblastoid cells of the mother (II-10) showed the abnormal X to be late-replicating in all cells.

A FISH study with the X-chromosome painting probe was carried out on chromosomes of the child's mother.

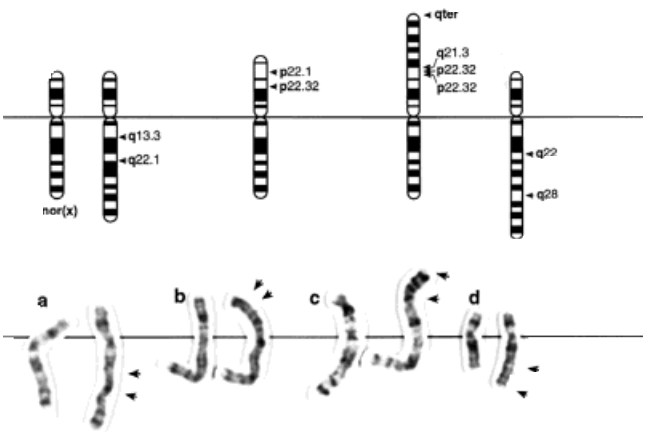

Fig. 3. G-banded partial karyotypes and idiogram of abnormal Xs. **a**: Patient 1. **b**: Female carrier of duplication seen in patient 2. **c**: Patient 3. **d**: Patient 4.

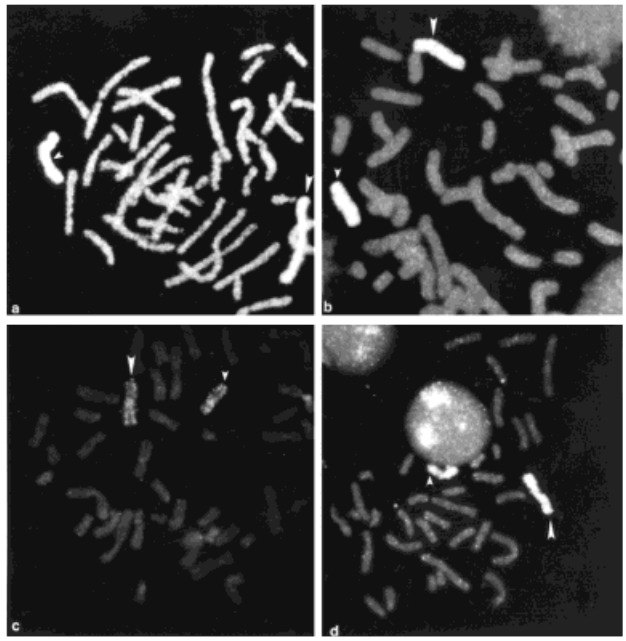

Fig. 4. Metaphases hybridized with X chromosome-painting probe. **a**: Patient 1. **b**: Patient 2. **c**: Patient 3. **d**: Patient 4. Large arrows indicate duplicated X, and small arrows indicate normal X.

This showed the extra segment to be X chromosome in origin (Fig. 4b). When GTG-banding suggested an Xp duplication, single copy probes RC8, DMD, and STS, mapping to Xp22.2, Xp21, and Xp22.32, respectively, were hybridized onto cells of the mother to establish extent of the duplication. RC8 and STS regions were duplicated (Fig. 5b,c) but DMD was not duplicated, suggesting that the duplication includes Xp22.32 and Xp22.2, but not Xp21. The extent and breakpoints of the duplicated X in this family were defined in combination with GTG-banded karyotypes as 46,inv dup(X)(p22.32→p22.1)Ymat.

Patient 3

Chromosome analysis from lymphocyte cultures suggested a 46,X,dup(X) karyotype (Fig. 3c). The length and GTG-banding pattern of the extra segment on the X allowed presumptive identification of its chromosomal origin as an X-chromosome duplication. The father's chromosomes were normal, but the mother was unavailable for study. Replication studies showed that the abnormal X was late-replicating in the patient.

FISH studies with an X-chromosome painting probe verified the X-chromosome origin of the entire duplication (Fig. 4c). Single-copy probes XIST, Cos9, DMD, and STS, mapping to Xq13.2, Xq28, Xp21, and Xp22.32, respectively, were used in lymphocytes of patient 3. FISH studies showed duplication of both Cos9 (Xq28) and STS (Xp23.32) sequences on the long and short arms, respectively, of the abnormal X (Fig. 5d,e). However, DMD was not duplicated. Both Cos9 and STS localized to the appropriate band on the normal X (Fig. 5d,e). Thus this duplication appeared to be a compound

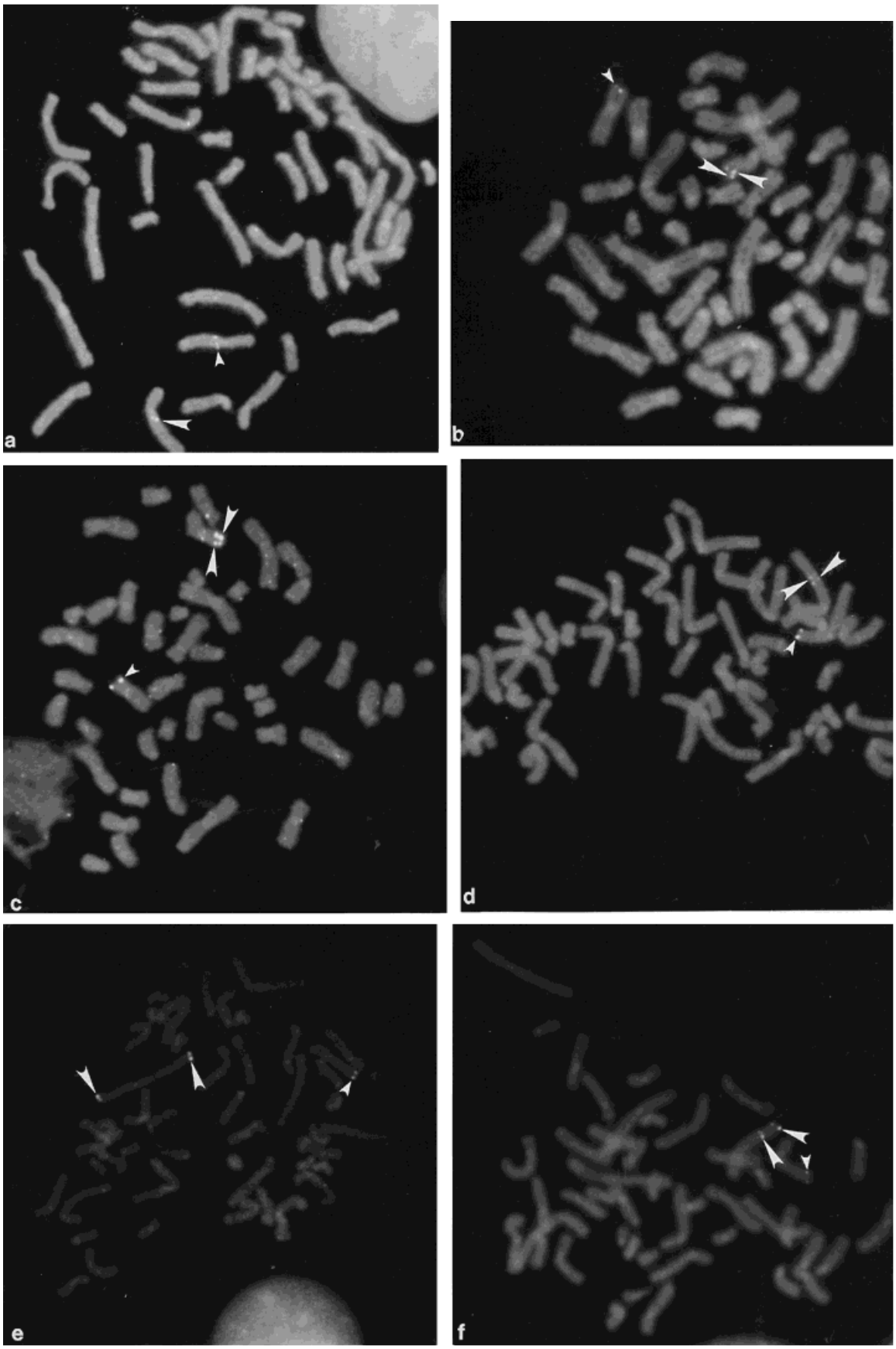

Fig. 5

duplication positioned on Xp and involving both Xp and Xq sequences. The other probes, XIST and DMD, appeared as single signals at Xq13.2 and Xp21 in both normal and abnormal Xs. Thus the karyotype of this patient was established by FISH plus GTG-banding as 46,X,dup dup(X) (qter→q21.3::p22.33→p22.32::p22.32→qter).

Patient 4

Chromosome analysis from lymphocyte cultures showed a 46,X,Xq+ karyotype. Partial GTG-banded karyotypes from lymphocytes are given in Figure 3d. Replication studies documented late replication of the abnormal X chromosomes. The patient's mother had normal chromosomes. The father was not available for study.

X-chromosome painting probe demonstrated the X chromosome origin of the extra segment (Fig. 4d). FISH with unique-sequence DNA probes XIST and Cos9, located at Xq13.2 and Xq28, respectively, was performed on metaphase chromosomes of patient 4. Only Cos9 showed duplicated signals in all cells (Fig. 5f). This suggests that the Cos9 locus was included in the duplicated segment in this patient, and that the breakpoint was distal to the XIST locus. In combination with GTG-banding, the karyotype of patient 4 was identified as 46,X,dup(X) (q22→q28).

DISCUSSION

Since the first case of a duplication on the short arm of the X chromosome was reported by Narahara et al. [1979], 37 cases of X chromosome duplications, including the current cases, were reported, of which 49% (18 of 37) were familial; 35% (13 of 37) were de novo (Tables I–III). Only 4 of 13 de novo patients were males; this may reflect the relative inviability of de novo X chromosome duplications in males compared to females. For most X-chromosome duplications, a prezygotic origin is suggested by the absence of a normal cell line and lack of mosaicism. However, in one case [Schwartz et al., 1986], a familial X-chromosome inverted duplication was traced to a grandparental mitotic error.

Aughton et al. [1993] suggested that the clinical effects of duplications of Xq reflect the extent of duplication. In phenotypically normal females the duplicated Xq segments were smaller and more proximal than those Xq duplications observed in females with clinical abnormalities. In combining these data with findings from other X duplications in males and females, the duplicated segments in individuals with abnormal phenotypes were found to be more frequently located on the distal region of Xp or on the proximal segment of Xq. The larger X duplications occurred exclusively in females (Tables I–III, Fig. 6). From Figure 6 it may be observed that the distribution of duplications in males and females was different. In females only three Xp duplications were ascertained, but nine were ascertained in males, and eight of nine of these cases were in the Xp2 region. There were more females with duplications in Xq than in Xp, and these mostly included Xq2. There were 10 duplications of Xq reported in males and these occurred both proximally and distally, except in a region from Xq24–Xq26 where no duplications have yet been reported.

As shown in Table II, surviving males with Xp duplication almost invariably have mental retardation, various minor anomalies, and small stature [Nielsen and Langkjaer, 1982; Cianchetti et al., 1992; Grubs et al., 1993]. Several cases of Xp duplication had sex reversal [Bernstein et al., 1980; Scherer et al., 1989; Arn et al., 1994; Bardoni et al., 1994], the X-duplication in all these cases involving Xp21. The gene SRVX (sex reversal X), recently mapped to Xp21.2→p22.11 by Arn et al. [1994], was suggested to be part of a pathway of sex-determining genes. Rao et al. [1994] suggested that this region includes a second X gene involved in regulation of testis determination located at Xp21. Subsequently, Bardoni et al. [1994] identified a locus DSS (dosage-sensitive sex reversal gene) in a 160-kb region of Xp21. Two active copies of this locus result in sex reversal in males. In our patient 2 with a karyotype of 46, inv dup(X)(p22.32→p22.1)Y, the duplication as defined by FISH seems not to include Xp21 and the patient was not sex-reversed; nonetheless, the genitalia were abnormal, with undescended right testis and shawl scrotum. Similar findings were present in his male first cousin with the same Xp duplication. Since neither of these males were biopsied, we can only suggest that there might be additional genes in this region affecting sex differentiation.

Patient 2 is similar to two other cases with an X-duplication of p22.1→p22.3 [Narahara et al., 1979; Grubs et al., 1993]. The duplication of the Xp22.1→p22.32 region in patient 2 was established by FISH to have one copy of DMD and two copies of STS and RC8, while the patient of Grubs et al. [1993] had one copy of ZFX and two copies of STS. In the patient of Narahara et al. [1979], the karyotype was based on banding studies alone. All 3 patients shared common findings: mental retardation, developmental delay, and short stature, although minor anomalies appeared distinct in each patient (Table II).

The clinical findings of the female patients reported in this paper may be compared to patients previously reported (Table III). Case 1 (46,X,dup(X)(q22.1→q13.3)) and a case reported by Carrio et al. [1991] had a similar duplicated segment (Xq13→q22), but these could not be compared clinically as their patient was aborted. Some of the manifestations of patient 1 are found in Thode-Leonard syndrome, as seen in male patients [Thode et al., 1988].

Patient 3 had clinical findings of hypotonia, growth failure, and developmental delay found in other females with Xq duplications (Table III). The limited

Fig. 5. FISH images of metaphases after use of single-copy probes. Large arrows indicate duplicated X, and small arrows indicate normal X. **a**: Patient 1. Single signal with XIST probe in both Xs. **b**: Patient 2. Double signals on duplicated X and single signal on normal X. RC8 probe. **c**: Patient 2. Double signals on duplicated X and single signal on normal X. STS probe. **d**: Patient 3. Double signals on duplicated X. STS probe. **e**: Patient 3. Double signals on duplicated X and single signal on normal X. Cos9 probe. **f**: Patient 4. Two signals on duplicated X and single signal on normal X. Cos9 probe.

TABLE I. Clinical Findings in Male Patients With Xq Duplications*

Case	Karyotype	MR	FD	DD	HG	SS	FA	Hyp	Other	References
1	46,dup(X)(q13→q22)Y,mat	+	+	+	+	+	+	+	Cryptorchidism, frog-like posture, scoliosis, simian creases, clinodactyly	Steinbach et al., 1980
2	46,inv dup(X)(q21→q24)Y,mat	+	+	+	NR	+	+	+	Cryptorchidism, lethargic	Schwartz et al., 1986
3	46,dup(X)(q13→q21.1)Y,mat	+	+	+	NR	+	+	NR	Inguinal hernia, lethargic, bent knee, delayed bone age	Thode et al., 1988
4	46,dup(X)(q13.3→q21.2)Y,mat	+	+	+	+	+	+	+	Cryptorchidism, simian creases, empty sella syndrome	Yokoyama et al., 1992
5	46,dup(X)(q12→q21.1)Y	+	NR	+	NR	+	NR	+	Macrocephaly	Muscatelli et al., 1992
6	46,dup(X)(q13→q22)Y	+	+	+	NR	+	+	+	Cryptorchidism, inguinal hernia, abnormal myelinization, abnormal EEG, delayed bone age	Cremers et al., 1987
7	46,dup(X)(q13.1→q21.2)Y,mat	+	NR	NR	+	+	+	+	Testes unpalpable, lethargic, simian creases, bent knee	Vejerslev et al., 1985
8	46,dup(X)(q12→q13.2)Y,mat	+	NR	+	NR	+	+	NR	NR	Schmucker et al., 1993
9	46,dup(X)(q26.3→qter)Y,mat	+	NR	NR	NR	+	NR	+	Severe malformations	Mohandas et al., 1987
10	46,dup(X)(q12→q22)Y,mat	+	NR	NR	NR	NR	NR	NR	NR	Schmidt et al., 1991
11	46,dup(X)(q26.3→qter)Y,mat	+	NR	+	+	+	+	+	Cryptorchidism, inguinal hernias	Vasquez et al., 1995

*DD, developmental delay; FA, facial anomalies; FD, feeding difficulties; HG, hypoplastic genitalia; Hyp, hypotonia; mat, maternal; MR, mental retardation; NR, not reported; SS, small stature, +, present.

clinical follow-up of this patient prevents further clinical comparison.

Patient 4, with a duplication of Xq22→q28, experienced recurrent miscarriages. She also had mild mental retardation, learning difficulties, speech defect, social problems, and some minor anomalies, such as microcephaly, high-arched palate, clinodactyly, small hands and feet, and very short stature. Most of these findings were similar to those of of a 12-year-old girl with a similar X duplication (Xq22→q28) (Table III) [see also Crandall et al., 1993]. In addition, the patient of Crandall et al. [1993] had body asymmetry and optic nerve hypoplasia. Although we were not able to define precisely the proximal breakpoint of the duplication in patient 4 using unique sequence probes, clinical findings and results of GTG-banding suggest the similarity of the duplicated segments in these 2 patients.

Both dup(Xp) and dup(Xq) chromosomes were observed in a number of females with minor anomalies ranging from mild to significant [Knuutila et al., 1984; Aughton et al., 1993; Varella-Garcia, 1981; Deng et al., 1990; Wyandt et al., 1991; Crandall et al., 1993; Yu et al., 1993], including the 3 female patients presented in this paper. These 3 patients and most of those in the literature showed late-replicating patterns of the duplicated X (Table III). However, some phenotypically normal and abnormal females with X duplications may demonstrate early-replicating patterns in their duplicated X chromosomes. Magenis et al. [1984] reported on a malformed and developmentally delayed female infant with a karyotype suggestive of inv dup(X) (q26.3→qter), with the abnormal X chromosome active in all 109 cells examined. Tuck-Muller et al. [1993] reported on an 11-year-old girl who had minor anomalies and shared short stature, mental retardation, and a karyotype of dic inv dup(X)(qter→p22.3::p22.3→cen:) with her mother. The daughter's duplicated X was early-replicating in 11% of her lymphocytes and late-replicating in all of her fibroblasts. Her mother's duplicated X was consistently late-replicating. In the case re-

TABLE II. Clinical Findings in Male Patients With Xp Duplications*

Case	Karyotype	MR	SS	DD	FA	Other	Reference
12	46,dup(X)(p22.1→p22.32)Y,mat	+	+	+	+	FTT, undescended testis, shawl scrotum, bilateral hearing loss, optic nerve atrophy of the right eye, clinodactyly, hypertonia, abnormal EEG	Present patient 2
13	46,dup(X)(p22.1→p22.3)Y,mat	+	+	+	+	Hypotonia, FTT, slight hemisphere atrophy	Narahara et al., 1979
14	46,dup(X)(p22.1→p22.3)Y	+	+	+	+	Autism, mild hypospadias	Grubs et al., 1993
15	46,dup(X)(p22→pter)Y,mat	+	+	+	+	Conjunctival telangiectasia	Cianchetti et al., 1992
16	46,dup(X)(p21.2→p22.3)Y	+	NR	NR	+	Sex reversal, hypoplastic external genitalia, bilateral simian creases, hypotrophy of muscle	Scherer et al., 1989
17	46,dup(X)(p21.1→p22.2)Y	+	NR	+	+	Sex reversal, camptodactyly, hypotonia	Scherer et al., 1989
18	46,dup(X)(p21→pter)Y,mat	+	NR	NR	NR	Cleft palate, pulmonary stenosis, micropenis	Just et al., 1993
19	46,dup(X)(p21→pter)Y,mat	+	+	+	NR	Sex reversal, cleft palate, CHD, FTT, liver dysfunction, hypercholesterolaemia, hypotonia, asymmetrical head	Bernstein et al., 1980
20	46,dup(X)(p11.2→p21.2)Y,mat	+	+	+	NR	Obese, macrocephaly, kyphosis, hypotonia, CHD, abnormal EEG	Nielsen and Langkjaer, 1982
21	46,dup(X)(p21.2→p22.3)Y	+	NR	NR	NR	Sex reversal, multiple minor anomalies	Bardoni et al., 1994
22	46,dup(X)(p21.2→p22.2)Y	+	NR	NR	NR	Sex reversal, multiple minor anomalies	Bardoni et al., 1994
23	46,dup(X)(p21.2→p22.1)Y	+	NR	NR	NR	Sex reversal, multiple minor anomalies	Bardoni et al., 1994
24	46,dup(X)(p21.2→p22.11)Y,mat	NR	+	+	+	Incomplete sex reversal	Arn et al., 1994

*CHD, congenital heart disease; DD, developmental delay; EEG, electroencephalogram; FA, facial anomalies; FTT, failure to thrive; mat, maternal; MR, mental retardation; NR, not reported; SS, short stature; +, present.

ported by Arn et al. [1994], the mother was phenotypically normal, although the duplicated X was early-replicating in 20 of 66 cells (30.3%). In the current study, the mother of patient 2 showed a mildly abnormal phenotype and late replication was also demonstrated for her duplicated X chromosome.

Wyandt et al. [1991] and Aughton et al. [1993] have postulated that cytogenetic replication studies cannot distinguish phenotypically normal and abnormal females with X duplications. Other explanations for the phenotypic manifestations in female carriers of X duplications have been suggested by Van Dyke et al. [1983]. These state that: 1) random inactivation of the paternal- and maternal-derived X chromosomes may be essential in some tissues; 2) equal doses of genes on Xp and Xq may be essential for normal development; 3) two structurally normal X chromosomes may be essential for normal gonadal function; 4) there may be unrecognized mosaicism for 45,X or other cell lines; 5) the normal X might be inactivated in some cells, leading to a functional excess of genes in the duplicated region; and 6) a position effect might alter the interrelationships among genes. Aughton et al. [1993] considered that imprinting might be involved. Preferential inactivation of the duplicated X chromosome (derived from one parent) implies preferential activation of the normal X chromosome (derived from the other parent), and phenotypic consequences might occur either through an imprinting phenomenon or through the expression of recessive genes on the active normal X chromosome.

A further possibility which might be considered is that the duplicated segment may contain genes which normally escape inactivation [Hall, 1991]. Such genes are located in the distal and proximal short arm and the proximal long arm of the X chromosome [Disteche, 1995]. However, only 1 of the 3 clinically abnormal females reported here carries a duplication in any of these regions (patient 1, dupX(q13.3→q22), which would suggest that the presence of noninactivated genes in the duplication are not responsible for phenotypic effects. Both of the remaining duplications in clinically-affected females are distally located on Xq (patient 3, Xq21.3→qter; patient 4, Xq22→Xq28). The presence of a second pseudoautosomal region near the Xq telomere has been reported [Freije et al., 1992], but genes escaping inactivation located in this region have not been identified.

The application of FISH with chromosome-specific or unique DNA sequence probes has been shown to be useful in identifying rearrangements of chromosomes, including X-chromosome duplications [Schmucker et al., 1993; Just et al., 1993; Grubs et al., 1993; Tuck-Muller

TABLE III. Clinical Findings and Late Replication of X Chromosomes in Female Cases*

Case	Karyotype	MR	SS	DD	FA	Others	Late X	Reference
25	46,X,inv dup(X)(q13.3→q22.1)	NR	−	+	+	Club feet, meningo-myelocele, feeding difficulty, hypotonia	dup(X) in L	Present patient 1
26	46,X,dup dup(X)(qter→q21.3::pter→p22.32::p22.32→qter)	+	+	+	+	Hypotonia, small hands with displaced thumbs	dup(X) in L	Present patient 3
27	46,X,dup(X)(q22→q28)	+	+	−	−	Multiple miscarriages, learning problems, speech defect, social problems, microcephaly, clinodactyly	dup(X) in L	Present patient 4
28	46,X,dic inv dup(X)(p22.3→cen)mat	+	+	+	+	Seizures, motor and speech delay	89% dup(X) in L; 100% dup(X) in F	Tuck-Muller et al., 1993
29	46,X,dup(X)(p21→p22.1)	−	+	NR	±	Secondary amenorrhea, small uterus, myopia, some Ullrich-Turner signs, abnormal EEG, epilepsy, dyslexia	dup(X) in L	Wyandt et al., 1991
30	46,X,dup(X)(p11.4→p22.1)	+	NR	NR	+	NR	NR	Deng et al., 1990
31	46,X,dup(X)(q13→qter)	−	+	+	+	Seizures, microcephaly, hypotonia	dup(X) in L	Aughton et al., 1993
32	46,X,inv dup(X)(q28-q22)	−	+	NR	NR	Limb asymmetry, microcephaly, optic nerve hypoplasia, learning difficulties, behavior problems	dup(X) in L	Crandall et al., 1993
33	46,X,dup(Xq)	NR	+	+	+	Whole-body hemihyper-trophy, growth failure, camptodactyly	dup(X) in L	Yu et al., 1993
34	46,X,dir dup(X)(q21→q27)mat	+	−	NR	+	Hypoplastic external genitalia, small uterus, high level of FSH and LH, asymmetric limbs, camptodactyly	dup(X) in L	Varella-Garcia, 1981
35	46,X,dup(X)(q21.1→q24)	+	+	+	+	Small uterus, myopia, right hemiplegia, abnormal EEG, acrocephaly, hypotonia, hyperflexible joints	dup(X) late in L	Knuutila et al., 1984
36	46,X,inv dup(X)(q26.3→qter)	+	NR	+	NR	"Dysmorphic"	dup(X) in L	Magenis et al., 1984
37	46,X,inv dup(X)(q13.3→q27.2)	−	+	NR	+	Gonadal dysgenesis, secondary amenorrhea, diabetes	dup(X) in L	Van Dyke et al., 1983
11	46,X,dup(X)(q26.3→qter)mat	+	+	NR	NR	NR	57% dup(X) in L	Vasquez et al., 1995

*DD, developmental delay; EEG, electroencephalogram; FA, facial anomalies; F, fibroblasts; FSH, follicle-stimulating hormone; L, lymphocytes; LH, leutinizing hormone; mat, maternal; MR, maternal retardation; NR, not reported; SS, small stature; +, present; −, absent.

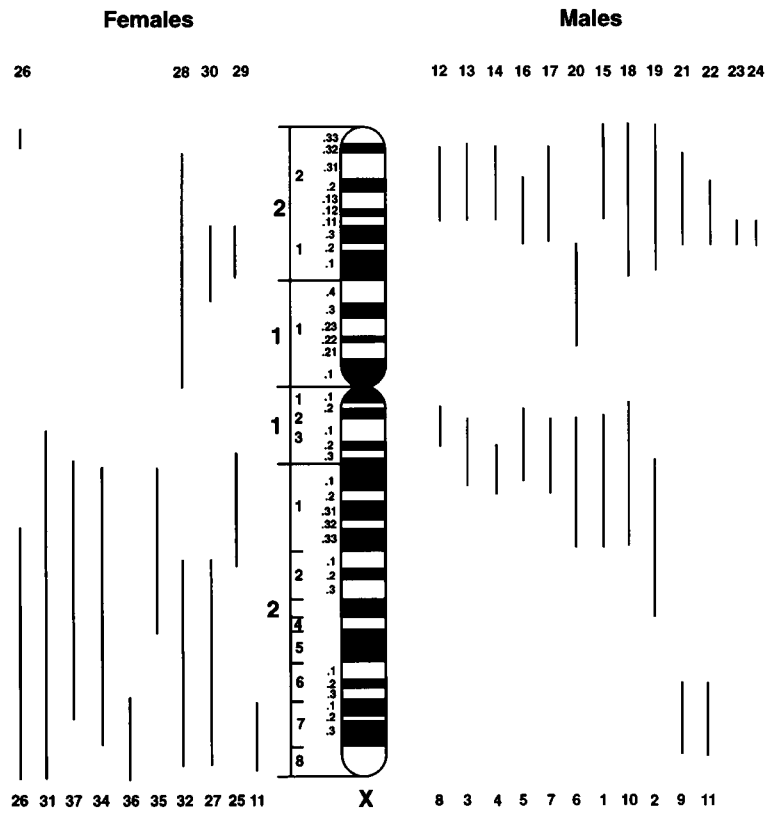

Fig. 6. Ideogram showing extent of duplicated segment in current patients and those reported in the literature. **Left**: Females. **Right**: males. The numbering of patients is the same as shown in Tables I–III.

et al., 1993]. We have demonstrated four X duplications. A combination of FISH and standard cytogenetic analysis was necessary to define the extent of each duplication. In each of our patients, the identification of an abnormal X chromosome as a duplicated X was initially established by using an X-chromosome-painting probe. Studies with single-copy sequence probes STS, RC8, XIST, Cos9, and DMD, combined with reexamination of GTG-banding, defined the orientation of breakpoints of duplicated segments. This is particularly clear in patient 3, in whom a compound duplication which originated from both Xp and Xq was not detected until a series of FISH studies was performed.

One interesting finding (in patient 3) was the presence not only of a duplication of Xq (demonstrated by the Cos9 probe), but the presence of a second small duplication in the proximity of the breakpoint demonstrated by duplication of STS (Xp22.32), but not present for DMD (Xp21).

In reviewing such unexpected findings in de novo inversions, Gordon and Halliday [1995] proposed that strand misalignment at DNA repair might result in deletion or duplication of sequences at the site of breakage. Such an interpretation, if extended to include de novo duplications, might account for the findings in patient 3.

REFERENCES

Arn P, Chen H, Tuck-Muller C, Mankinen C, Wachtel G, Li S, Shen C, Wachtel S (1994): SRVX, a sex reversing locus in Xp21.2→p22.11. Hum Genet 93:389–393.

Aughton DJ, Al Saadi AA, Johnson JA, Transue DJ, Trock GL (1993): Dir dup(X) (q13–qter) in a girl with growth retardation, microcephaly, developmental delay, seizures, and minor anomalies. Am J Med Genet 46:159–164.

Bardoni B, Zanaria E, Guioli S, Floridia G, Worley KC, Tonini G, Ferrrante E, Chiumello G, McCabe ERB, Fraccaro M, Zuffardi O, Camerino G (1994): A dosage sensitive locus at chromosome Xp21 is involved in male to female sex reversal. Nat Genet 7:497–501.

Bernstein R, Jenkins T, Dawson B, Wagner J, Dewald G, Koo GC, Wachtel SS (1980): Female phenotype and multiple abnormalities in sibs with a Y chromosome and partial X chromosome duplication: H-Y antigen and Xg blood group findings. J Med Genet 17: 291–300.

Carrio A, Soler A, Perez MT, Margarit E, Salami C, Fuster J, Cararach J, Farguell T, Fortuny A (1991): Prenatal diagnosis of dup (X)(q13q22) in CVS. Clin Genet 40:128.

Cianchetti C, Muntoni F, Falchi A, Nucaro A, Sannio-Fancello G, Cao A, Marrosu M (1992): X-linked mental retardation and characteristic physical features in two brothers with duplication Xp22–Xpter. Am J Med Genet 43:475–478.

Crandall BF, Lamb AN, Estabrooks LL (1993): A girl with an inverted duplicated Xq and an abnormal phenotype. Am J Hum Genet 53:540.

Cremers FPM, Pfeiffer RA, van de Pol TJR, Hofker MH, Krise TA, Ropers HH (1987): An interstitial duplication of the X chromosome in a male allows physical fine mapping of probes from the Xq13–q22 region. Hum Genet 77:23–27.

Deng H, Xia J, Ishikawa M, Nikawa N (1990): Parental origin and mechanism of formation of X chromosome structural abnormalities: Four cases determined with RFLPs. Jinrui Idengaku Zasshi 35:245–251.

Disteche C (1995): Escape from X inactivation in human and mouse. Trends Genet 11:17–22.

Freije D, Helms C, Watson MS, Donis-Keller H (1992): Identification of a second pseudoautosomal region near Xq and Yq telomeres. Science 258:1784–1787.

Gordon AJ, Halliday JA (1995): Inversions with deletions and duplications. Genetics 140:411–414.

Grubs R, Klinepeter K, Stewart W, Hayworth R, Jewett T, Thomas IT, Rao PN, Pettenati MJ (1993): Duplication Xp in a male as confirmed by molecular-cytogenetics: Case report and review. Am J Hum Genet 53:554.

Hall JG (1991): X inactivation of the X chromosome is not complete. Growth 7:9.

Just W, Vogel W, Llerena JR, Vargas FR, Martins RR, de Almeida JCC (1993): Xp duplication (p21–pter) in a patient with Y chromosome, micropenis, congenital anomalies but an unambiguous male external genitalia. Am J Hum Genet 53:1583.

Knuutila S, Heinonen K, Hongell K, Varonen S, Simell O (1984): A duplication within the critical fertility region of X chromosome in a mentally retarded woman with normal menarche. Hereditas 101: 253–255.

Latt SA, Willard HF, Gerald PS (1976): BrdU-33258 Hoechst analysis of DNA replication in human lymphocytes with supernumerary or structurally abnormal X chromosomes. Chromosoma 57:135–153.

Magenis RE, Donlon TA, Canfield WM, Koler R, Bigley R (1984): Partial duplication of X long arm, q26.3–qter: Gene dose effect of G6PD, HPRT, factor VIII:c, and factor IX. Cytogenet Cell Genet 37:528.

Mohandas T, Geller RL, Yen PH, Rosendorff J, Bernstein R, Yoshida A, Shapiro LJ (1987): Cytogenetic and molecular studies on a recombinant human X chromosome: Implication for the spreading of X chromosome inactivation. Proc Natl Acad Sci USA 84:4954–4958.

Muscatelli F, Verna JM, Philip N, Moncla A, Mattei MG, Fontes M (1992): Physical mapping of an Xq-proximal interstitial duplication in a male. Hum Genet 88:691–694.

Narahara K, Kodama Y, Kimura S, Kimoto H (1979): Probable inverted tandem duplication of XP in a 46,Y,Xp+ boy. Jinrui Idengaku Zasshi 24:105–110.

Nielsen KB, Langkjaer F (1982): Inherited partial X chromosome duplication in a mentally retarded male. J Med Genet 19:222–224.

Pinkel D, Landegent J, Collins C, Fuscoe J, Segraves R, Lucas J, Gray J (1988): Fluorescence *in situ* hybridization with human chromosome-specific libraries: Detection of trisomy 21 and translocations of chromosome 4. Proc Natl Acad Sci USA 85:9138–9142.

Rao PN, Klinepeter K, Stewart W, Hayworth R, Grubs R, Pettenati MJ (1994): Molecular cytogenetic analysis of a duplication Xp in a male: Further delineation of a possible sex influencing region on the X chromosome. Hum Genet 94:149–153.

Scherer G, Schempp W, Baccichetti C, Lenzini E, Bricarelli FD, Carbone LDL, Wolf U (1989): Duplication of an Xp segment that includes the ZFX locus causes sex inversion in man. Hum Genet 81:291–294.

Schmidt M, Sart DD, Kalitsis P, Leversha M, Dale S, Sheffield J, Toniolo D (1991): Duplications of the X chromosome in males: Evidence that most parts of the X chromosome can be active in two copies. Hum Genet 86:519–521.

Schmucker B, Cohen M, Schuffenhauer S, Genzel O, Bach I, Stengel-Rutkowski S, Meindl A (1993): Breakpoint analysis, XIST expression and X-inactivation study in an Xq proximal interstitial duplication involving the X inactivation center of a female carrier and her two sons with malformation syndrome. Am J Hum Genet 53:600.

Schwartz S, Schwartz MF, Panny SR, Peterson CJ, Waters E, Cohen MM (1986): Inherited X-chromosome inverted tandem duplication in a male traced to a grandparental mitotic error. Am J Hum Genet 38:741–750.

Steinbach P, Horstmann W, Scholz W (1980): Tandem duplication dup(X)(q13q22) in a male proband inherited from the mother showing mosaicism of X-inactivation. Hum Genet 54:309–313.

Thode A, Partington MW, Yip MY, Chapman C, Richardson VF, Turner G (1988): A new syndrome with mental retardation, short stature, and an Xq duplication. Am J Med Genet 30:239–250.

Tuck-Muller CM, Martinez JE, Batista DAS, Kearns WG, Wertelecki W (1993): Duplication of the short arm of the X chromosome in mother and daughter. Hum Genet 91:395–400.

Van Dyke D, Miller MJ, Weiss L (1983): The origin of inverted tandem duplications, and phenotypic effects of tandem duplication of the X chromosome long arm. Am J Med Genet 15:441–450.

Varella-Garcia M (1981): Structural aberration of the X chromosome in a patient with gonadal dysgenesis: An approach to karyotype-phenotype correlation. J Med Genet 18:228–231.

Vasquez AI, Rivera H, Bobadilla L, Crolla JA (1995): A familia Xp+ chromosome, dup(Xq26.3→qter). J Med Genet 32:891–893.

Vejerslev LO, Rix M, Jespersen B (1985): Inherited tandem duplication dup(X)(q13.1–q21.2) in a male proband. Clin Genet 27:276–281.

Wyandt HE, Bugeau-Michaud L, Skare JC, Milunsky A (1991): Partial duplication of Xp: A case report and review of previously reported cases. Am J Med Genet 40:280–283.

Yokoyama Y, Narahara K, Tsuji K, Moriwake T, Kanzaki S, Murakami M, Namba H, Ninomiya S, Higuchi J, Seino Y (1992): Growth hormone deficiency and empty sella syndrome in a boy with dup(X)(q13.3–q21.2). Am J Med Genet 42:660–664.

Yu CW, Curry CJR, Favour A, Immken L, Yu LC (1993): Replication study of an abnormal X chromosome by chromosome painting and BrdU/thymidine incorporation. Am J Hum Genet 53:630.